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秋水仙花生物碱*

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摘要 从云南昭通引种欧洲秋水仙花中分离出6个生物碱: 秋水仙碱 (Ⅰ), 2-去甲秋水仙碱 (Ⅱ), 2-去甲脱羰秋水仙碱 (Ⅲ), 2-去甲-17-羟基秋水仙碱 (Ⅳ), 2-去甲-β-光秋水仙碱 (Ⅴ), β-光秋水仙碱 (Ⅵ)。

关键词 秋水仙, 生物碱, 秋水仙碱
分类号 Q 946

Alkaloids from the Flowers of *Colchicum autumnale*

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Abstract This paper reports six alkaloids, colchicine(Ⅰ), 2-demethylcolchicine(Ⅱ), 2-demethyldemecolcine(Ⅲ), 2-demethylcolchifoline(Ⅳ), 2-demethyl-β-lumicolchicine(Ⅴ), and β-lumicolchicine(Ⅵ) isolated from the flowers of *Colchicum autumnale* which was introduced from Europe to Zhaotong, Yunnan, China.

Key words *Colchicum autumnale*, Alkaloids, Colchicine

Colchicine (Dewar, 1945), a tropholone alkaloid, is a principal alkaloid that was isolated from *Colchicum autumnale* (Liliaceae). It has a variety of medical purpose in the treatments of goat, breast cancer, skin cancer, leukemia and Hodgkin's disease. Thus much attention was paid to colchicine and its analogues for their strong anticancer activity and special structures (Jitak *et al*, 1993; Brossi, 1990).

This paper firstly reports the chemical constituents in the flowers of *Colchicum autumnale* which was introduced from Europe to Zhaotong, Yunnan. China. Six alkaloids were separated from the flowers. They were colchicine (Ⅰ), 2-demethylcolchicine(Ⅱ), 2-demethyldemecolcine(Ⅲ), 2-demethylcolchifoline(Ⅳ), 2-demethyl-β-lumicolchicine(Ⅴ), and β-lumicolchicine(Ⅵ). They were identified by using IR, ^{1}H NMR, ^{13}C NMR and MS.

The research results showed the content to colchicine(Ⅰ) in the flowers was low, and the content of 2-demethyldemecolcine(Ⅲ) was high.

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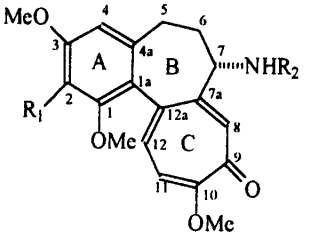
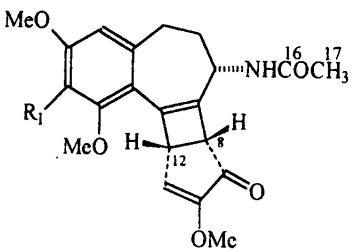


I $R_1=OCH_3$, $R_2=COCH_3$
II $R_1=OH$, $R_2=COCH_3$
III $R_1=OH$, $R_2=CH_3$
IV $R_1=OH$, $R_2=COCH_2OH$



V $R_1=OH$
VI $R_1=OCH_3$

RESULTS AND DISCUSSION

Compounds Ⅰ, Ⅱ, and Ⅵ were identified as colchicine (Baytop *et al*, 1980; Meksuriyen *et al*, 1988), 2 - demethylcolchicine)Hufford *et al*, 1979), and β - lumicolchicine (Meksuriyen, 1988; Chapman *et al*, 1963), respectively by comparison of their ^1H and ^{13}C NMR spectral date with those of authentic samples.

Compound Ⅲ $C_{20}H_{23}NO_5$, M 357. Its IR spectrum (3320 cm^{-1}) revealed the presence of hydroxyl group. The ^1H NMR (δ 4.01, 3.93, and 3.57) and ^{13}C NMR (δ 60.3 56.2, and 56.1) showed the presence of three methoxyl groups. A report (Hufford *et al*, 1979) suggested that the methoxyl groups at C - 10 and C - 3 can be assigned to the signals at δ 56.3 or δ 56.5, and those at C - 1 and C - 2 can be assigned to the signals at 61.3 or 61.5. So, Ⅲ is not 3 - demethyl or 10 - demethyl analogue, but 1 - demethyl or 2 - demethyl analogue. The ^1H NMR and ^{13}C NMR of C - 1, C - 2 and C - 3 of Ⅲ are similar to those of 2 - demethylcolchicine. Thus Ⅲ was a 2 - demthyl analogue of colchicine. But the difference Ⅲ from 2 - demethylcolchicine is the presence of $NHCH_3$(δ 2.28) and the missing $COCH_3$(none of the signals of CH_3 at δ 1.9 - 2.05 in ^1H NMR, and none of the signals of CO about δ 170.0 in ^{13}C NMR). So, Ⅲ was identified as 2 - demethyldemecolcine.

Compound Ⅳ $C_{21}H_{23}NO_7$, M 401. Its ^1H NMR spectrum (δ 3.95, 3.89, 3.58) and ^{13}C NMR spectrum (δ 56.3, 56.3 and 61.6) showed the presence of three methoxyl groups. Simmilar to Ⅲ, Ⅳ was a 2 - demethyl analogue of colchicine too. Its IR spectrum (1 635 cm^{-1}) showed the presence of - NHCOR at C - 7. However, there was no signals of $COCH_3$ in ^1H NMR, which indicated that R of - NHCOR was not methyl group. The ^{13}C NMR spectrum (δ 62.3) and the ^1H NMR spectrum (OH of δ 5.92, δ 4.12 and δ 3.92 of - CH_2O) showed the presence of - CH_2OH. Thus, the side chain of Ⅳ at C - 7 is - $NHCOCH_2OH$ group. Accordingly, the chemical structure of Ⅳ can be determined as 2 - demethylcolchifoline.

Compound Ⅴ $C_{21}H_{23}NO_6$, M 385. It was an isomer of compound Ⅱ. Its ^1H NMR spectrum (δ 3.94, 3.89, and 3.67) and ^{13}C NMR spectrum (δ 60.9, 56.8, 56.2) showed the presence of three

methoxyl groups. Compared with Ⅱ, Ⅴ was a 2 - demethyl analogue of colchicine also. However, it differed from Ⅱ in four methine (C_4, C_8, C_{11}, C_{12}). Its ^1H NMR spectrum [6.71(d, 1H), 6.50(s, 1H), 4.13(dd, 1H), 3.63(dd, 1H)], ^{13}C NMR(DEPT) spectrum [129.0(d), 108(d), 51.4(d), 43.1(d)], and IR spectrum (1718 cm^{-1}, 1640 cm^{-1}) indicated that Ⅴ was a lumicolchicine analogue. Furthermore, the signal at δ 4.82(m, 1H) was assigned to C - 7, which indicated Ⅴ was a β - lumicolchicine derivative (Meksuriyen *et al*, 1988). Accordingly, Ⅴ was identified as 2 - demethyl - β - lumicolchicine.

EXPERIMENTAL

General. Mps.: uncorr.; IR spectra were obtained on a IR - 450S spectrophotometer. NMR spectra were measured in $CDCl_3$ and recorded on a Brucker spectrospin AM - 400 spectrometer at 400 MHz for ^1H NMR and ^{13}C NMR(DEPT) using TMS as internal standard. EI - MS were measured on VG Autospec 3000 mass spectrometer at 70 eV accelerating voltate.

Plant material. The flowers of *Colchicum autumnale* L. were collected in Zhaotong, Yunnan, China and identified by Prof. Hu Zhi - Hao.

Extraction and isolation. The powder of dried flowers (600 g) was extracted with C_6H_6 in a Soxhlet apparatus, and the solvent was removed to afford a C_6H_6 extract. The extract was dissolved in H_2O. The H_2O layer was made acidified with 1% H_2SO_4 to pH 2.5, then extracted with Et_2O and $CHCl_3$ respectively. The $CHCl_3$ layer was evapd to dryness to give an acid fraction. The acid soln was basified with NH_4OH to pH 9 and extracted with $CHCl_3$ to give a basic fraction.

The acid fraction was separated on a silica gel column with eluting $CHCl_3$ by increasing amount of CH_3OH to give twelve fractions. The fifth fraction was further separated by preparative TLC on Al_2O_3 ($CHCl_3/CH_3OH = 70:1$), which obtained compound Ⅵ (6 mg). The sixth fraction was further separated by preparative TLC($CHCl_3/CH_3OH = 60:1$), which obtained compound Ⅰ (60 mg). The eighth fraction was further separated by preparative TLC($CHCl_3/CH_3OH = 40:1$), which obtained compound Ⅱ (57 mg) and Ⅴ (72 mg). The twelfth fraction was further separated by preparative TLC($CHCl_3/CH_3OH = 25:1$), which obtained compound Ⅳ (22 mg). The basic fraction was chromatographed on a silica gel column eluting with $CH_2Cl_2 - CH_3OH$(20:1) to afford compound Ⅲ (450 mg).

colchicine (Ⅰ): $C_{22}H_{25}NO_6$, yellow powder (AcOEt - Et_2O), mp 155 ~ 156℃. IRν_{max}^{KBr} cm^{-1}: 3430, 3300, 2950, 1640, 1618, 1590, 1560, 1490, 1250, 1100, 1020, 1020. EIMS m/z (%): 300(M^+, 57), 371(16), 356(100). ^1H NMR ($CDCl_3$): 8.50 (d, 1H, J = 6.1Hz, NH), 7.61(s, 1H, H - 8), 7.31(d, 1H, J = 10.9Hz, H - 12), 6.87(d, 1H, J = 10.9Hz, H - 11), 6.49(s, 1H, H - 4), 4.60(m, 1H, H - 7), 3.97(s, 3H, OCH_3 - 10), 3.91(s, 3H, OCH_3 - 2), 3.89 (s, 3H, OCH_3 - 3), 3.60(s, 3H, OCH_3 - 1), 1.90 ~ 2.50(m, 4H, CH_2 - 5 and CH_2 - 6), 1.92 (s, 3H, $COCH_3$). ^{13}C NMR data see Table 1.

2 - **demethylcolchicine** (Ⅱ): $C_{21}H_{23}NO_6$, yellow powder ($CHCl_3$), mp 210℃. IRν_{max}^{KBr} cm^{-1}:

3400, 2950, 1654. 1610, 1590, 1570. EIMS m/z(%): 385(M^+, 93), 356(94), 342(67), 298 (100). ^{1}H NMR ($CDCl_3$): 7.75(d, 1H, NH), 7.54(s, 1H, H-8), 7.30(d, 1H, J=10.8Hz, H-12), 6.83(d, 1H, J=10.8Hz, H-11), 6.48(s, 1H, H-4), 4.61(m, 1H, H-7), 3.97 (s, 3H, OCH_3-10), 3.89(s, 3H, OCH_3-3), 3.62(s, 3H, OCH_3-1), 1.85~2.50(m, 4H, CH_2-5 and CH_2-6), 1,93(s, 3H, $COCH_3$). ^{13}C NMR data see Talbe 1.

2-**demethyldemecolcine** (Ⅲ): $C_{20}H_{23}NO_5$, yellow powder (CH_2Cl_2-CH_3COCH_3), mp 124~125℃. IRν_{max}^{KBr}cm^{-1}: 3320, 2940, 2842, 1612, 1590, 1560, 1495, 1250. EIMS m/z (%): 357 (M^+, 100), 342(8), 328(23), 298(42), 193(92). ^{1}H NMR ($CDCl_3$, δ): 7.66(s, 1H; C_8-H), 7.29(d, 1H, J=10.7Hz; C_{12}-H), 6.84(d, 1H, J=10.7Hz; C_{11}-H), 6.53(2, 1H; C_4-H), 4.01(s, 3H; C_{10}-OCH_3), 3.93(s, 3H; C_3-OCH_3), 3.57(s, 3H; C_1-OCH_3), 3.37 (m, 1H; C_7-H), 1.71~2.47(m, 4H; C_5-CH_2 and C_6-CH_2), 2,28(s, 3H; $NHCH_3$). ^{13}C NMR data see Table 1.

2-**demethylcolchifoline** (Ⅳ): $C_{21}H_{23}NO_7$, yellow semi-solid. IRν_{max}^{KBr}cm^{-1}: 3400, 1635, 1600. EIMS m/z (%): 401(M^+, 77), 373(20), 342(57), 298(100). ^{1}H NMR($CDCl_3$): 7.74 (d, 1H, NH), 7.57(s, 1H; H-8), 7.31(d, 1H, J=10.8Hz; H-12), 6.84(d, 1H, J=10. 7Hz; H-11), 6.49(s, 1H;H-4), 5.92(1H, OH), 4.67(m, 1H, H-7), 4.12 and 3.98(2H, $COCH_2OH$), 3.95(s, 3H; OCH_2-10), 3.89(s, 3H; OCH_3-3), 3.58(s, 3H; OCH_3-1), 1.90 ~2.50(m, 4H, CH_2-5 and CH_2-6). ^{13}C NMR data see Table 1.

2-**demethyl-β-lumicolchicine** (Ⅴ): $C_{21}H_{23}NO_6$, colorless needles (CH_3OH), mp 225℃ (sublimation). IRν_{max}^{KBr}cm^{-1}: 3400, 1718, 1640, 1610, 1555, 1410. EIMS m/z (%): 385(M^+, 67), 371(26), 342(100), 328(57). ^{1}H NMR($CDCl_3$): 6.71(d, 1H, J=3.2, H-11), 6.50(s, 1H, H-4), 6.07(d, 1H, J=7.1Hz, NH), 4.82(m, 1H, H-7), 4.13(dd, 1H, J=3.2 and 2.8, H-12), 3.94(s, 3H, OCH_3-1), 3.89(s, 3H, OCH_3-3), 3.67(s, 3H, OCH_3-10), 3. 63(dd, 1H, J=2.8 and 2.0, H-8), 2.76(dd, 1H, J=15.8 and 7.9, H-5), 2.56(dd, 1H, J =15.8 and 9.0, H-5), 2.00(m, 2H, H-6), 2.04(s, 3H, $COCH_3$). ^{13}C NMR data see Table 1.

β-lumicolchicine (Ⅵ): $C_{22}H_{25}NO_6$, yellow powder (CH_3COCH_3-$CHCl_3$), mp 181~182℃: IRν_{max}^{KBr}cm^{-1}: 3380, 1725, 1630, 1605. EIMS m/z (%): 399(M^+, 15), 356(100). ^{1}H NMR(CD-Cl_3): 6.64(d, 1H, J=3.2, H-11), 6.45(s, 1H, H-4), 5.93(d, 1H, J=6.8Hz, NH), 4. 79(m, 1H, H-7), 4.08(dd, 1H, J=3.2 and 2.8, H-12), 3.94(s, 3H, OCH_3-1), 3.85(s, 3H, OCH_3-2), 3.83(s, 3H, OCH_3-3), 3.68(s, 3H, OCH_3-10), 3.59(dd, 1H, J=2.8 and 1.9, H-8), 2.73(dd, 1H, J=15.2 and 8.0, H-5), 2.58(dd, 1H, J=15.2 and 9.2, H-5), 2.05(s, 3H, $COCH_3$), 2.00(m, 2H, H-6). ^{13}C NMR data see Table.1

Table 1 ^{13}C NMR(DEPT) spectral data of compound Ⅰ - Ⅵ in $CDCl_3$

Assignment	Ⅰ	Ⅱ	Ⅲ	Ⅳ	Ⅴ	Ⅵ
C-1	151.1(s)	144.8(s)	144.2(s)	144.8(s)	145.3(s)	151.8(s)
C-2	141.6(s)	138.0(s)	138.8(s)	138.0(s)	137.6(s)	140.5(s)
C-3	153.5(s)	147.7(s)	147.4(s)	147.7(s)	147.1(s)	153.2(s)
C-4	107.4(d)	106.7(d)	106.5(d)	106.9(d)	108.1(d)	109.4(d)
C-5	29.8(t)	29.6(t)	29.6(t)	29.6(t)	31.6(t)	31.4(t)
C-6	52.7(d)	52.7(d)	62.6(d)	37.0(t)	32.3(t)	32.6(t)
C-7	52.7(d)	52.7(d)	62.6(d)	51.8(d)	51.4(d)	51.5(d)
C-8	130.4(d)	130.6(d)	131.0(d)	131.1(d)	51.4(d)	51.5(d)
C-9	179.6(s)	179.5(s)	179.7(s)	179.6(s)	200.8(s)	200.8(s)
C-10	164.0(s)	164.1(s)	163.8(s)	164.0(s)	157.8(s)	157.9(s)
C-11	112.9(d)	112.8(d)	112.0(d)	112.8(d)	129.0(d)	128.8(d)
C-12	135.5(d)	135.0(d)	131.9(d)	135.0(d)	43.1(d)	43.2(d)
C-1a	125.6(s)	125.0(s)	124.8(s)	125.0(s)	117.9(s)	117.8(s)
C-4a	134.2(s)	129.8(s)	134.5(s)	129.8(s)	126.1(s)	138.8(s)
C-7a	152.6(s)	152.2(s)	151.9(s)	151.6(s)	134.5(s)	137.5(a)
C-12a	137.0(s)	136.6(s)	137.5(s)	136.6(s)	136.8(s)	145.2(s)
C-1 OCH_3	61.4(q)	61.2(q)	60.3(q)	61.1(q)	60.9(q)	61.3(q)
C-2 OCH_3	61.2(q)	-	-	-	-	60.8(q)
C-3 OCH_3	56.1(q)	56.3(q)	56.2(q)	56.3(q)	56.2(q)	56.0(q)
C-10 OCH_3	56.3(q)	56.3(q)	56.1(q)	56.3(q)	56.8(q)	56.6(q)
$NHCH_3$	-	-	34.0(q)	-	-	-
$COCH_2OH$	-	-	-	62.3(t)	-	-
C-16	170.0(s)	169.9(s)	-	172.4(s)	170.3(s)	170.2(s)
C-17	22.6(q)	22.7(q)	-	-	23.4(q)	23.5(q)

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References

Baytop T, Sutlupinar N, Phillipson J D, 1980. Alkaloids from *Colchicum Turcicum*. *Planta Med*, **38** (3): 273 ~ 274

Brossi A, 1990. Bioactive Alkaloids. *J Med Chem*, **33**(9): 2311 ~ 2319

Chapman O L, Smith H G, King R W, 1963. The Structure of β - lumicolchicine. *J Am Chem Soc*, **85**: 803 ~ 806

Dewar M J S, 1945. Structure of Colchicine. *Nature*, **155**: 141 ~ 142

Hufford C D, Collins C C, Clark A M, 1979. Microbial transformations and ^{13}C NMR analysis of colchicine. *J Pharm Sci*, **68**(10): 1239 ~ 1243

Jitka U, Daniela W, Veronika L, *et al*, 1993. Biochemical evaluation of colchicine and related analogs. *Planta Med*, **59**(2): 144 ~ 147

Meksuriyen D, Lin L J, Cordell G A, 1988. NMR studies of colchicine and its photoisomers, β - and γ - umiocolchicines. *J Nat Prod*, **51**(1): 88 ~ 93